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Asplenia Syndrome in a Child With a Balanced Reciprocal Translocation of Chromosomes 11 and 20 [46,XX,t(11;20)(q13.1;q13.13)]

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We present a 6-year-old girl with a balanced 11;20 translocation [46,XX,t(11;20)(q13.1; q13.13)pat], asplenia, pulmonic stenosis, Hirschsprung disease, minor anomalies, and mental retardation. This case represents the second report of an individual with situs abnormalities and a balanced chromosome rearrangement involving a breakpoint at 11q13. Polymerase chain reaction (PCR) analysis of microsatellite markers excluded uniparental disomy for chromosomes 11 and 20. Segregation analysis of markers in the 11q13 region in the proposita and her phenotypically normal carrier sibs did not show a unique combination of maternal and paternal alleles in the patient. We discuss several possible explanations for the simultaneous occurrence of situs abnormalities and a balanced 11;20 translocation. These include (1) chance, (2) a further chromosome rearrangement in the patient, (3) gene disruption and random situs determination, and (4) gene disruption plus transmission of a recessive or imprinted allele from the mother.

KEY WORDS: asplenia, translocation, chromosome 11, chromosome 20, heterotaxy, situs inversus, Hirschsprung disease, pulmonary stenosis

INTRODUCTION

In the egg and early embryo, the dorsoventral, anterior-posterior, and left-right axes are formed sequentially. Left-right determination occurs before organ formation and results in asymmetrical positioning of the developing heart, lungs, liver, spleen, and gastrointestinal tract. In approximately one in 10,000 individuals, situs solitus, the normal left-right orientation, is replaced by its mirror image, situs inversus (totalis), in which the heart and spleen are on the right and the liver is on the left. Complete situs inversus usually does not impair function because all organs are present, although their left-right orientation is reversed.

Other abnormalities of symmetry have a variety of descriptors including situs ambiguous, heterotaxy, isomerism, laterality sequence, Ivemark syndrome, and polyasplenia syndrome [Burn, 1991; Ivemark, 1955]. In these conditions there is loss of asymmetry; that is, the sides of the body are mirror images of each other. Bilateral right-sidedness, also called Ivemark or asplenia syndrome, results in bilateral tri-lobed lungs, two right atria, asplenia, a central liver, and gastrointestinal anomalies including bowel malrotation, Hirschsprung disease, and imperforate anus [Freedom, 1972; Rose et al., 1975; Mishalany et al., 1982]. Bilateral left-sidedness, also termed polysplenia, results in such features as bilateral bilobed lungs, two left atria, and multiple spleens [Van Mierop et al., 1972; Peoples et al., 1983; Burn, 1991]. The heart defects and other abnormalities seen in bilateral right- and left-sidedness often result in clinically significant disease.

The genetics of the laterality syndromes remains largely unknown, although some individuals with complete situs inversus have Kartagener syndrome, an autosomal recessive disorder of the dynein arms of cilia, resulting in bronchiectasis, male sterility, and anosmia [Kartagener and Stucki, 1962; Afzelius, 1976]. In the mouse, the autosomal recessive gene *iv* results in situs inversus (totalis) or varying degrees of heterotaxy [Hummel and Chapman, 1959; Layton et al., 1993; Seo et al., 1992]. The gene has been mapped to mouse chromosome 12 [Brueckner et al., 1989; Hanzlik et al., 1990], in a region homologous to the telomeric region of human chromosome 14, but no candidate gene has been identified in the mouse. A second murine gene, *inv*, has been mapped to chromosome 4 by insertional mutagen-

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esis and 100% of the homozygous offspring of two heterozygous transgenic mice display situs inversus or heterotaxy [Yokoyama et al., 1993]. The region of chromosome 4 containing the transgenic insertion site is homologous to human chromosomes 6q and 9q.

In humans, it is also likely that several genes are involved in situs determination. Examples of autosomal recessive, dominant, and X-linked inheritance have been documented [Burn, 1991; Casey et al., 1993; Mikkila et al., 1994]. The occurrence of bilateral right-sidedness (asplenia) and bilateral left-sidedness (polysplenia) within the same sibship suggests that these are manifestations of the same genetic defect and, thus, the term polyasplenia is frequently used to describe these conditions [Zlotogora and Elian, 1981; Opitz, 1985].

Chromosome rearrangements are often useful in indicating areas of the genome where particular genes may be located. Several chromosome abnormalities have been reported in individuals with situs abnormalities. These include single reports of either balanced or unbalanced rearrangements of chromosomes 2, 8, 10, 12, 13, 18, and 22 [Wilson et al., 1991; Carmi et al., 1992; Genuardi et al., 1993; Koiffmann et al., 1993]. There are also reports of balanced rearrangements involving a break at 7q22 in individuals with situs abnormalities in association with split hand/split foot syndrome [Genuardi et al., 1993; Koiffmann et al., 1993]. Fukushima et al. [1993] identified an infant with polysplenia syndrome and a balanced paracentric inversion of chromosome 11 with breaks at q13 and q25. In the present report we describe a child with asplenia and a balanced 11;20 translocation with a similar breakpoint at 11q13. Such multiple, independent occurrences of situs abnormalities involving the same chromosome region may be especially useful in directing the search for genes involved in axis determination.

CLINICAL REPORT

The proposita was delivered at term to a 31-year-old woman after a pregnancy complicated by hypertension, edema, and poor weight gain. Birth weight was 3,700 g. When seen by us at age 6 years her weight was between the 25–50th centile, her height was between the 10–25th centile, and her head circumference was at the second centile. Her eyes were deep-set and wide-spaced with epicanthal folds. She had medially flaring eyebrows, low-set, posteriorly angulated ears, and a prominent lower jaw. She was mildly hirsute and had long, tapering fingers and flat feet. She had undergone a balloon valvuloplasty for pulmonic stenosis. Abdominal defects included asplenia and Hirschsprung disease requiring bowel resection and a colostomy. She was moderately to severely mentally retarded. An MRI of the head at age 3 years was normal. In infancy she was found to have a balanced 11;20 translocation inherited from her clinically normal father.

The proposita's family includes 6 sibs, 4 of whom inherited the same paternal 11;20 translocation (Table II). All sibs are physically healthy including the oldest, a chromosomally normal daughter with a history of acute lymphocytic leukemia. Two children with the balanced translocation have learning disabilities, but there is no mental retardation. The patient's mother does not have a history of pregnancy losses.

CYTOGENETIC AND MOLECULAR FINDINGS

Chromosome studies of the proposita, her father, and her sibs were repeated in our laboratory using routine and synchronized culture methods on peripheral blood lymphocytes. At a resolution of 700 bands, the proposita's 46,XX,t(11;20)(q13.1;q13.13) translocation (Fig. 1) was determined to be balanced and identical to the arrangement in her father and her 4 carrier sibs. In addition, we found the paternal grandmother and her other two offspring to be translocation carriers.

We analyzed polymerase chain reaction (PCR)-amplified microsatellite markers on chromosomes 11 and 20 in the proposita, her grandparents, parents, and sibs. DNA was extracted from blood using a standard phenol-chloroform method or a QIAmp kit (Qiagen, Inc., Chatsworth, CA). ^{32}P-labelled PCR products were resolved on a sequencing gel and bands were visualized by autoradiography.

The results excluded uniparental disomy for both chromosomes involved in the translocation (Table I). Five chromosome 11 loci in the 11q13 region were used to study the segregation of markers in the proposita and her sibs. Four of the 5 markers were informative and allowed us to determine which allele the mother transmitted to each of her carrier and noncarrier offspring (Table II). There was no locus at which the proposita received a different maternal allele from all of her clinically normal, carrier sibs. That is, for each locus, at least one of the other carrier sibs received the same maternal allele as the proposita.

DISCUSSION

Wilson et al. [1991] offered several possible explanations for an association between situs abnormalities and familial, balanced chromosome rearrangements. These included (1) chance, (2) a further, undetected chromosome rearrangement in the proband, (3) gene disruption at the chromosome breakpoint resulting in random situs determination with only a proportion of translocation carriers being affected, (4) gene disruption with transmission of a recessive mutation or an imprinted allele from the other parent, and (5) transection of a maternal effect gene.

TABLE I. Uniparental Disomy Studies

Chromosome 11	Location	FA[a]	MO	PR	Chromosome 20	Location	FA	MO	PR
D11S922	11p15.5	AC	BD	CD	RPN2	20q12-13.1	BB	AC	BC
PYGM	11q13.1	BB	AD	BD					

[a]FA, father; MO, mother; PR, proband. Letters A-E designate PCR products of different sizes.

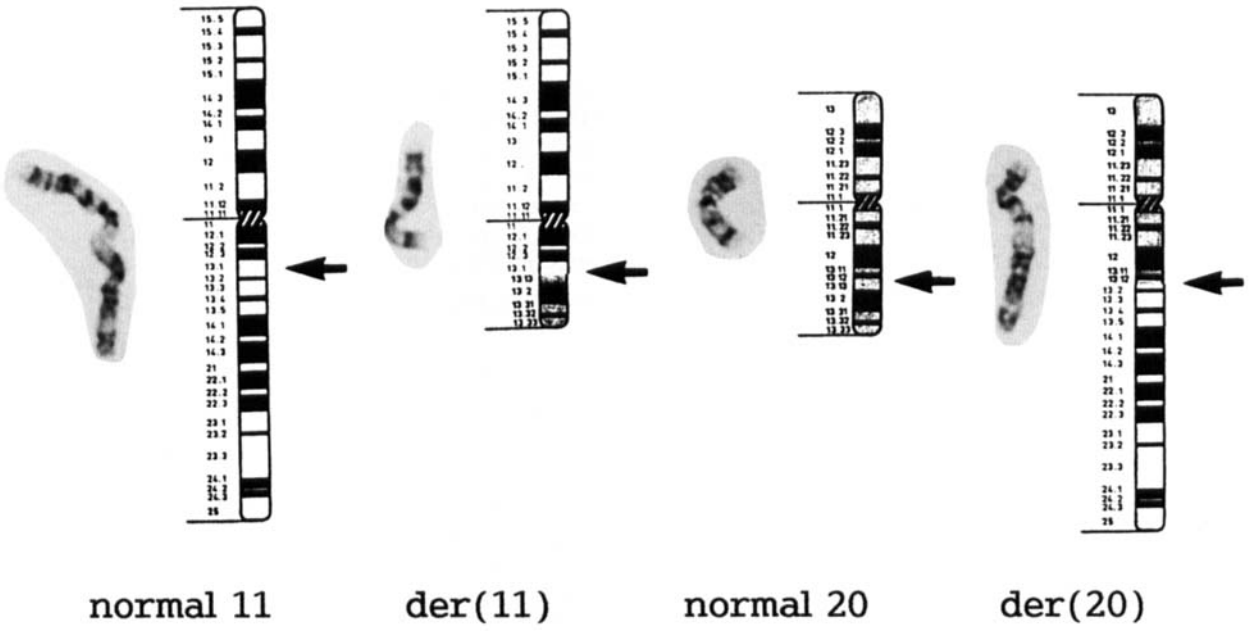

Fig. 1. Partial karyotype of the proband showing t(11;20)(q13.1;q13.13). Breakpoints are indicated by arrows.

These alternatives can be examined in the present case. In doing so we focus on chromosome 11 rather than on chromosome 20 because ours is the second report of laterality abnormalities with a breakpoint at 11q13. These two reports increase the likelihood that the association between the translocation and the situs abnormalities is not due to chance.

While a further chromosome rearrangement in the proband cannot be ruled out, extended banding did not reveal any observable differences between the proposita and the other translocation carriers.

Gene disruption resulting in random situs determination is also unlikely. While diagnostic studies were not obtainable on family members, all translocation carriers except the proposita are healthy. It is unlikely that only one individual out of the nine translocation carriers would be clinically affected if transection of a dominant gene at the translocation breakpoint resulted in random situs determination.

Fukushima et al. [1993] postulated that imprinting might explain why their proband with a balanced paracentric inversion of chromosome 11 had polysplenia, whereas the proband's carrier father did not. In the mouse, there is evidence that the distal portion of chromosome 7, the region syntenic with human 11q13, is imprinted [Searle and Beechey, 1985; Bartolomei and Tilghman, 1992]. However, little is known about the extent of the imprinting or the particular genes affected. If a gene or genes at 11q13 is important in situs determination, we think it unlikely that imprinting is involved because it cannot explain why, of 5 carrier sibs, only one is affected. In addition, we ruled out uni-

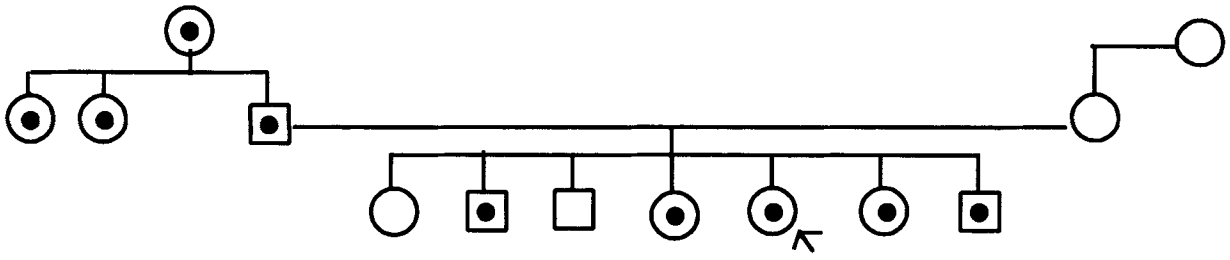

TABLE II. Segregation of Chromosome 11 Markers

Locus	Location	I-1	II-3	III-1	III-2	III-3	III-4	III-5	III-6	III-7	II-4	I-2
PYGM	q13.1	BA	BB	BD	BD	BA	BA	BD	BD	BA	DA	AE
D11S480	q13.1	AB	AC	CC	AC	CC	AC	AC	AC	AC	CC	CA
D11S956	q13.1	BE	BC	CD	BA	CA	BA	BD	BD	BA	DA	AB
INT2	q13.3	BD	BA	AD	BD	AC	BC	BD	BD	BC	DC	CB
D11S527	q13.5	CA	CD	DB	CD	DD	CD	CD	CB	CB	BD	DB

⊙, □: Carriers of balanced translocation. Letters A-E designate PCR products of different sizes.

parental disomy, a phenomenon which, when present in a subject with growth abnormalities, suggests an imprinted region of the genome.

Wilson et al. [1991] also suggested the possibility of maternal effect genes influencing embryonic symmetry. While an intriguing theory in their maternally derived 12;13 translocation, it is not applicable to the present, paternally inherited translocation.

A remaining possibility is that a gene at the 11q13 breakpoint has been disrupted by the translocation and, in addition, the mother is heterozygous for an abnormal allele at the same locus which she has passed to the proposita but not to any of her other four carrier offspring. The chance for this distribution of alleles to occur is one in 32 or approximately 3%. Our studies did not find evidence for this alternative in the segregation pattern of several chromosome 11 markers. However, molecular characterization of the 11q13 breakpoint relative to known genes at that location should be done to identify possible candidate genes whose integrity and segregation pattern could then be studied in depth.

The list of candidate genes for situs determination includes the proto-oncogenes and other developmental genes which map to several of the chromosome regions associated with laterality abnormalities [Wilson et al., 1991]. Wilson pointed out that the *HOX*3 cluster of homeotic genes and int-1 (also termed Wnt-1), a member of the gene family implicated in embryonic axis development [Parr and McMahon, 1994], are located in the region of the 12q13 breakpoint. However, he subsequently reported that he had provisionally excluded int-1 gene as a candidate gene in the family with the 12;13 translocation [Wilson, 1992].

Developmentally important genes in the 11q13 region which should be considered as possible candidate genes include the fibroblast growth factors, FGF-3 (int-2) and FGF-4. Embryonic axis formation and inductive activities have been attributed to members of this gene family [Tannahill et al., 1992; Niehrs and De Robertis, 1992]. Although our preliminary analyses did not disclose differences in int-2 alleles between the proposita and her sibs, we suggest that clarification of the translocation breakpoints and further analysis of *int*-2 and other candidate genes in the 11q13 region are necessary. Developmentally important genes are widely dispersed throughout the genome, and, if they have a role in axis determination, it would explain why abnormalities in laterality are observed with a number of different chromosome rearrangements.

A further approach to identifying genes important in laterality determination would be to perform linkage analyses on asplenia kindreds specifically focusing on areas of the chromosomes such as 11q13 where breaks have been found in isolated individuals with situs abnormalities. Traditional linkage studies have already pointed to Xq24-q27.1 as the location of a gene for situs determination [Casey et al., 1993].

In conclusion, it is possible that the association between the 11;20 translocation and laterality disturbances in our proposita is due to chance, particularly since she is the only carrier in the family who is affected and we could find no cytogenetic or DNA evidence to link the two occurrences. However, the fact that this is the second report of laterality abnormalities in association with a translocation involving a breakpoint at 11q13 increases the likelihood that a gene or genes on chromosome 11 may be involved in determining laterality. Karyotyping of other individuals with situs abnormalities should be done to identify additional chromosome rearrangements and strengthen the case for examining particular chromosome regions in detail.

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